Preprint
UCRL-JC-150806

Functionalized Silicon Membranes for Selective Bio-Organisms Capture

S. E. Letant, B. R. Hart, A. W. van Buuren, L. T. Terminello

This article was submitted to Spring MRS 2003 Meeting, San Francisco, CA, April 21 – 25, 2003

January 9, 2003

Functionalized silicon membranes for selective bio-organisms capture

Sonia E. Létant*
Bradley R. Hart
Anthony W. van Buuren
Louis J. Terminello

Chemistry and Materials Science Directorate
Lawrence Livermore National Laboratory
Livermore, California 94550, USA

* e-mail: letant1@llnl.gov

Membranes with various pore size, length, morphology and density have been synthesized out of diverse materials[1-5] for size exclusion-based separation. An example of application is the sterilization of intravenous lines by exclusion of bacteria and viruses using Polyvinylidene Fluoride membranes with 0.1 μm diameter pores. The need for chemically specific filtration has recently been addressed, but for small molecules only[6-10]. An important problem remaining to be solved is the selective capture of large bio-organisms for decontamination or analysis of air and liquids such as drinking water and body fluids. To achieve this goal, materials with controlled pore diameter, length and surface chemistry are required. In this letter, we present the first functionalized silicon membranes and demonstrate their ability to selectively capture simulated bio-organisms. These extremely versatile and rigid devices open the door on a new class of materials able to recognize the external fingerprints of bio-organisms such as size and outer membrane proteins for specific capture and detection applications.

Preparation of macropores in n-type silicon using the back-side illumination technique was first reported by Lehmann and Föll[11] in 1990 and since then, most of the research has been devoted to photonics applications[12, 13] where pores are blind holes etched in a thick silicon wafer. Loncar et al.[14] published a silicon photonic crystal made out of a thin

silicon slab (0.3 μm) suspended in air in which they prepared through holes with a 0.4 μm diameter by ion beam lithography. This technique is well adapted to photonics applications, where thin suspended slabs of material are required to improve the confinement of photons in the vertical direction. But it does not allow the fabrication of longer through pores required by sensing applications to improve the probability of organism or molecule capture.

We prepared through pores on pre-patterned n-type silicon wafers using the back-side illumination technique described by Lehmann et al.[11] In this method, the top of the wafer is patterned with inverted pyramids by standard lithography. The purpose of these top pits is to concentrate the electric field and to constitute nucleation sites for the pore growth (see Fig. 1a and 1c). We added a back pattern which plays a key role in the membrane fabrication by both providing high robustness to the devices as well as membrane thickness tunability. As it can be seen in Fig. 1b, 25 back windows were prepared by lithography followed by potassium hydroxide (KOH) directional etch to define 25 membrane areas per sample. The initial wafer thickness of 545 μm was thinned down to 45 μm under the back windows, as shown on Fig. 1d. The thickness of the silicon slab remaining under the back windows defines the membrane thickness and is controlled by the KOH etch time. The advantage of this design is that it combines thin silicon membrane areas with a thick silicon support grid. It can be pointed out that none of the membranes etched on back patterned wafers cracked or broke during the experiments.

The pre-patterned samples were etched in an electrochemical cell in an aqueous solution of hydrofluoric acid (HF). The positive charges required to dissolve silicon in this electrolyte were photo-generated on the back of the n-type wafer and concentrated at the tips of the top nucleation pits by applying a voltage across the sample (see methods for details). The pore diameter can be tuned by controlling the number of positive charges collected at the pit tips, which depends on the intensity of the back light illumination. The pore length is proportional to the duration of the HF etch. Though the entire top surface of the samples was patterned with nucleation sites, pores grew only in the thinned areas, where the electric field is the most intense. The pore growth rate was measured to be 0.25 ± 0.05 μm /min. Etching pre-patterned wafers thinned down to 15 μm for one hour lead

to the formation of through pores in the back windows, as shown on Fig. 2f. With this etching technique, the membrane pore diameter was tuned between 0.5 and 2.0 μm by increasing the light intensity in order to generate a photocurrent between 200 and 500 pA/pore.

The absence of holes and cracks in the membranes, as well as their size exclusion ability, were checked by mounting the devices in a U-tube permeation cell fitted with a sealed piston and by measuring the fluorescence of feed and permeate solutions of size calibrated dyed micro-beads forced through the pores (data not shown). Seven aqueous bead solutions with diameters ranging between 0.040 and 4.0 μm were used. A good correlation between the membrane cut off determined by the bead solution assay and the pore diameter measured by SEM was observed, indicating sharp pore size distributions (see Fig. 2a and 2e) and perforation free devices.

We have shown that, contrary to most of the commercial membranes, silicon membranes prepared by electrochemistry on pre-patterned wafers present controlled pore position, diameter and length and that they are good size exclusion filters above 0.5 μm. Additionally, silicon constitutes a material of choice because its surface can be derivatized with various functional groups in order to add chemical specificity to membrane pore walls. A recent review article by Buriak[15] recapitulates the different approaches explored by various research groups. Basically, functionalized alkyl chains can be covalently anchored on silicon surfaces either via a Si-O-Si-C attachment, or, via a direct Si-C attachment. The first procedure requires an oxidation of the silicon surface prior to its functionalization,[16] while the second procedure starts on a silicon hydride surface. The Si-C attachment can be catalyzed by a Lewis acid[17], light[18] or temperature[19]. We chose the Lewis acid catalyzed reaction because it was demonstrated to stabilize porous silicon samples in very demanding conditions such as boiling in aerated water and in aqueous KOH at pH=10[17] as well as in simulated human blood plasma[20]. We recently showed that this technique can be used to anchor biotin on porous silicon surfaces by an extension of the linker[21]. The procedure used for biotin is general to the covalent attachment of any kind of bio-molecule (e.g. any accessible lysine site can be attached to

the linker). This reaction also works on flat silicon surfaces. We functionalized flat silicon surfaces with biotin and incubated them in a solution of streptavidin-coated micro-beads for 45 min. After rinsing extensively with deionized water, about 40% of the area of the functionalized samples was coated with a monolayer of micro-beads and no beads where found on unfunctionalized reference surfaces. This result is in agreement with the 20-30% coverage rates reported for Lewis acid catalyzed attachments on porous silicon[15].

We performed the same functionalization on the silicon membranes (see methods for details). Wetting problems when starting the functionalization on pores covered with hydrophobic silicon hydride were avoided with our procedure because none of the reaction steps involves aqueous solutions. Fig. 3 shows the result of a flow through experiment performed on a biotin functionalized silicon membrane with a top pore diameter of 2.0 μm and 200 nm diameter beads. When the uncoated beads are pushed through the membrane, the fluorescence of the permeate solution is identical to the fluorescence of the feed solution, showing that no beads are captured in the pores. When streptavidin-coated bead with the same diameter are pushed through the membrane at the same speed, the fluorescence signal of the permeate solution is decreased by 40 ± 4 %. The number of beads captured in the membrane pores can be extracted from the concentrations of the feed and permeate solutions (corrected from the deionized water flow through rinses) and divided by the number of pores. The average number of beads captured per pore is found to be about 43 beads per pore. This number is well correlated with the number of 38 beads trapped per pore counted by SEM on cross sections of the functionalized membranes performed after the flow though experiments. An SEM picture of streptavidin-coated micro-beads anchored on the walls of a pore is provided in insert in Fig.3. The deficit of beads observed by SEM is due to beads attached on the top surface of the sample. Almost no beads where observed on the bottom surface of the sample because the silicon nitride mask was kept on the support grid to prevent biotin functionalization and because the interaction time between the liquid and the bottom surface is short. It is not possible to keep the top silicon nitride mask on because silicon nitride dissolves during the HF electrochemical etch.

The 40% trapping efficiency obtained for a pore to bead diameter ratio of 10 could be optimized by adjusting the physical parameters of the membrane such as the pore diameter, length and density. But a decrease in the flow rate and an increase of the probability of pore clogging at high bead concentration and smaller pore diameters should be expected. To place the trapping efficiency obtained into perspective, we can reference a recent article by Lee et al.[8], in which they report a transport selectivity coefficient of 2.6 for their enantioselective bio-nanotube membrane (the selectivity coefficient being the ratio of the fluxes of the RS to the SR enantiomers of the drug 4-[3-(4-fluorophenyl)-2-hydroxy-1-[1,2,4]triazol-1-yl-propyl]-benzonitrile permeating through the device). This selectivity coefficient, obtained for a pore diameter of 0.035 μm, was increased to 4.5 by reducing the pore diameter to 0.020 μm, but at the price of a significant analyte flux reduction.

We showed that rigid silicon membranes with adjustable thickness and pore size could be prepared and covalently functionalized. Biotin functionalized membranes specifically capture streptavidin-coated beads, letting uncoated beads through. We believe that this experiment constitutes a proof of concept for the selective capture of bio-organisms such as viruses and bacteria which have a size comparable to the size of the micro-beads used in the present work and which differ from one another by specific outer membrane proteins, simulated by the streptavidin anchored on the bead surface. The next step in our research will be to convert these membranes into detectors, able to measure the amount of organisms captured in the field and in real time for counter-terrorism applications as well as for water surveillance or body fluid analysis.

METHODS

MEMBRANE PRE-PATTERNING

N-type, Phosphorus doped, (100) oriented silicon wafers with a resistivity of 2 Ω.cm and an initial thickness of 545 μm were pre-patterned on both sides by standard contact photolithography. A thin layer of silicon nitride (0.2 μm) was grown on the surface of the silicon wafers by low-pressure chemical vapor deposition (LPCVD) and the top and

bottom patterns were defined by plasma etch. The top pattern consists of 2 x 2 μm squares fully etched with potassium hydroxide (KOH) in order to form pyramid shaped pits. These pits are arranged into a square lattice with a 4 μm period. They constitute nucleation sites for the growth of the membrane pores. The bottom pattern consists of 250 x 250 μm square windows etched in KOH in order to thin the silicon wafer from 545 down to 45 μm. Each sample is a 1 x 1 cm square, entirely patterned with pyramidal pits on the top and patterned with 25 back windows.

MEMBRANE ETCHING

Silicon membranes were prepared by back-side, light assisted electrochemical etch of pre-patterned silicon samples. The samples were mounted in an electrochemical cell and connected to a potentiostat. An ampere meter was introduced in the circuit to measure the photocurrent. The electrolyte solution was a mixture of de-ionized water (97.5% per volume) and hydrofluoric acid (HF) (2.5% by volume). A drop of surfactant (Ilfotol™) was added for 250 ml of solution prepared. The counter electrode was a platinum coil immersed in the electrolyte and the work electrode was connected to the sample via gallium-indium eutectic paste, which provides an ohmic contact. The positive charges required for the dissolution of silicon in the presence of HF were created on the back of the sample by the white light from a tungsten lamp. The light was filtered with a BG-12 filter transmitting wavelengths between 250 and 500 nm to stop deep penetrating infra-red light. The light intensity was varied in order to generate a photocurrent between 200 to 500 pA/pore. A 1.5 V bias was applied across the sample in order to generate an electric field, which localizes the electrochemical dissolution reaction at the tip of the nucleation pits.

For the functionalized membranes, the silicon nitride top and bottom masks were kept on the samples to prevent derivatization and consequently, to reduce binding of the micro-beads on the top and bottom surface. Contact was taken on the back of the sample by removing the nitride on a small area and rubbing the gallium-indium eutectic paste. The nitride covering the top area exposed to the electrolyte solution during the membrane fabrication was removed prior to starting the electrochemical reaction by a 30 min exposure to an aqueous solution of HF (25% by volume) in the electrochemical cell.

MEMBRANE FUNCTIONALIZATION

The hydride terminated silicon membranes were functionalized with biotin using a five step procedure including hydrosilylation, reduction of the surface bound nitrile, attachment of SPDP (*N*-succinimidyl 3-(2-pyridyldithio)propionate), reductive cleavage of pyridyl disulfide protecting group, and finally, attachment of biotin. Hydrosilylation of the silicon membranes was carried out in a round-bottom Schlenk type flask equipped with a single 24/40 taper joint and glass stopcock controlled side-arm. The samples were placed in the flask, which was then sealed with a septum and purged with dry, oxygen-free nitrogen gas. 5-Hexynenitrile (0.10 mL, 0.95 mM) was added to the samples followed by the addition of a 1.0 M hexane solution of $EtAlCl_2$ (150 μL, 0.15 mmol). After 3 h at room temperature, the membranes were washed under a nitrogen atmosphere with THF, followed by CH_2Cl_2 and then EtOH. They were then dried under vacuum. Reduction of the surface bound nitrile to a 1° amine was achieved by adding a 1.0 M diethyl ether solution of $LiAlH_4$ (150 μL, 0.15 mmol). After 30 min at room temperature, the membranes were washed under a nitrogen atmosphere with THF (3 x 5 mL), followed by CH_2Cl_2 (3 x 5 mL) then EtOH (3 x 5 mL) then dried under a nitrogen stream followed by vacuum. The amino functionalized membranes were immersed in a solution of SPDP (2 mg, 6.4×10^{-3} mmol) in DMF under nitrogen and left to react for 3 h with occasional agitation. The remaining SPDP solution was removed and the samples were rinsed with DMF (3 × 5 mL) followed by ethanol (3 x 5 mL) then dried under a nitrogen stream followed by vacuum. The SPDP functionalized membranes were immersed in a solution of dithiothreitol (DTT) (15.4 mg, 0.1 mmol) in 10% $EtOH/H_2O$ (10 mL) and left to react for 1 h with occasional agitation. The remaining DTT solution was removed and the parts were rinsed with fresh 10% $EtOH/H_2O$ (3 × 5 mL) then EtOH (5 × 5 mL) then dried under a nitrogen stream followed by vacuum. The sulfhydryl samples were immersed in a solution of GMBS (*N*-(γ-maleimidobutyryloxy)succinimide)) (2.0 mg, 7.1×10^{-3} mmol) in DMF under nitrogen and left to react for 3 h with occasional agitation. The remaining GMBS solution was removed and the samples were rinsed with DMF (3 × 5 mL) followed by ethanol (3 x 5 mL) then dried under a nitrogen stream followed by vacuum. The NHS-ester membranes were immersed in a solution of Biotin cadaverine (5.0 mg,

0.011 mmol) in DMF under nitrogen and left to react overnight with occasional agitation. The remaining solution was removed and the samples were rinsed with DMF (5 × 5 mL) followed by ethanol (5 x 5 mL) then dried under a nitrogen stream followed by vacuum.

MEMBRANE CHARACTERIZATION

The samples were characterized by Field Emission Scanning Electron Microscopy (FE-SEM) with a Hitachi S-4500 microscope. Samples were mounted on the sample holder using carbon tape and graphite paint. SEM images were taken with an acceleration voltage between 3 and 6 KeV. Both backscattered and secondary electron images were acquired.

MEMBRANE PERMEATION

Uncoated polystyrene dyed beads with diameters ranging from 0.04 to 4.0 μm and streptavidin-coated polystyrene dyed beads with a diameter of 0.2 μm were purchased from Bangs Laboratories Inc. For the membrane permeation experiments, bead solutions containing 10^7 beads/ml and 0.001% of Triton X in de-ionized water were prepared. Triton X was added for wetting purposes. Each membrane was mounted into a U-tube diffusion chamber, with feed solution on one side and atmospheric pressure on the other side. A sealed piston was used to push 5 ml of feed solution through the membrane at a flow rate of 0.5 $ml.cm^{-2}.min^{-1}$. Alternatively, the membranes were sealed with Al tape and parafilm on a metallic support grid and inserted into the stainless steel filter holder of a Luer syringe with Teflon O-rings. The fluorescence of the feed and permeate solutions was measured with a Perkin Elmer fluorimeter model LS45 to assess the bead concentration in each solution. After each of the experiments, 5ml of deionized water were pushed through the membrane at a speed of 0.5 $ml.cm^{-2}.min^{-1}$ to flush adsorbed beads out of the pores. The fluorescence data from the permeate solution were corrected from this dilution by a factor 2.

References

1. Idris, A., Ismail, A. F., Noordin, M. Y. & Shilton, S. J. *Journal of membrane science* **205**, 223-237 (2002).
2. Martin, C. R. *Science* **266**, 1961-1966 (1994).
3. Jirage, K. B., Hulteen, J. C. & Martin, C. R. *Science* **278**, 655-658 (1997).
4. Desai, T. A., Hansford, D. & Ferrari, M. *Journal of Membrane Science* **159**, 221-231 (1999).
5. Leoni, L., Boiarski, A. & Desai, T. A. *Biomedical Microdevices* **4**, 131-139 (2002).
6. Jirage, K. B., Hulteen, J. C. & Martin, C. R. *Anal. Chem.* **71**, 4913-4918 (1999).
7. Bok Lee, S. & Martin, C. R. *Chem. Mater.* **13**, 3236-3244 (2001).
8. Lee, S. B., Mitchell, D. T., Lacramioara, T., Nevanen, T. K., Soderlund, H. & Martin, C. R. *Science* **296**, 2198-2200 (2002).
9. Fernandez-Lopez, S., Kim, H.-S., Choi, E. C., Delgado, M., Granja, J. R., Khasanov, A., Kraehenbuehl, K., Long, G., Weinberger, D. A., Wilcoxen, K. M. & Ghadiri, M. R. *Nature* **412**, 452-455 (2001).
10. Chun, K.-Y. & Stroeve, P. *Langmuir* **18**, 4653-4658 (2002).
11. Lehmann, V. & Foll, H. *J. Electrochem. Soc.* **137**, 653-659 (1990).
12. Birner, A., Wehrspohn, R. B., Gosele, U. M. & Busch, K. *Adv. Mater.* **13**, 377-388 (2001).
13. Lehmann, V., Stengl, R., Reisinger, H., Detemple, R. & Theiss, W. *Appl. Phys. Lett.* **78**, 589-591 (2001).
14. Loncar, M., Doll, T., Vuckovic, J. & Scherer, A. *Journal of Lightwave Technology* **18**, 1402-1411 (2000).
15. Buriak, J. M. *Chem. Rev.* **102**, 1271-1308 (2002).
16. Dancil, K.-P. S., Greiner, D. P. & Sailor, M. J. *J. Am. Chem. Soc.* **121**, 7925-7930 (1999).
17. Buriak, J. M. & Allen, M. J. *J. Am. Chem. Soc.* **120**, 1339-1340 (1998).
18. Stewart, M. P. & Buriak, J. M. *J. Am. Chem. Soc.* **123**, 7821-7830 (2001).
19. Boukherroub, R., Morin, S., Wayner, D. D. M., Bensebaa, F., Sproule, G. I., Baribeau, J.-M. & Lockwood, D. J. *Chem. Mater.* **13**, 2002-2011 (2001).
20. Canham, L. T., Reeves, C. L., Newey, J. P., Houlton, M. R., Cox, T. I., Buriak, J. M. & Stewart, M. P. *Adv. Mater.* **11**, 1505-1507 (1999).
21. Hart, B. R., Létant, S. E., Kane, S. R., Hadi, M., Shields, S. J. & Reynolds, J. G. *Chem. Comm.*, accepted for publication, (2003).

Acknowledgements

This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48. It was funded by a Laboratory Directed Research and Development grant (LDRD-ER # 00-ERD-009).

Correspondence and requests for materials should be addressed to S.E.L.

Competing financial interests

The authors declare that they have no competing financial interests.

Figure Captions

Figure 1

Pre-patterned silicon sample. **a**, optical top view, **b** optical bottom view, **c** SEM top view and insert with detail of one pyramidal pit, **d** SEM cross section and insert with detail of two pyramidal pits.

Figure 2

Silicon membranes. **a-d** SEM pictures of a silicon membrane with a thickness of 15 μm and a pore diameter of 2 μm: **a** top view, **b** cross section, **c** bottom view and **d** bottom view in a corner of the back window showing the edge of the membrane area. **e-f** SEM pictures of a silicon membrane with a thickness of 45 μm and a pore diameter of 0.5 μm: **e** top view, **f** cross section, **g** bottom view and **h** bottom view in a corner of the back window showing the edge of the membrane area. One can note that the pore diameter enlarges toward the end of the etch (the bottom of the pore) due to an increased number of positive charges at the back of the sample. Different rows of pores are shown in the cross sections, due to the difficulty to cleave the samples across the back grid.

Figure 3

Selective capture of simulated bio-organisms.

Luminescence of feed and permeate solutions of uncoated (left) and streptavidin-coated (right) micro-beads pushed through a biotin functionalized silicon membrane with a top pore diameter of 2 μm and a thickness of 45μm. Both kinds of beads have a 200 nm diameter (schemes are not to scale). The uncoated beads are dyed with a green dye and the streptavidin-coated beads are dyed with an orange dye. The plain lines correspond to the feed solutions and the dashed lines correspond to the permeate solutions. An SEM cross section of streptavidin beads captured in a biotin functionalized pore is shown in insert.

Figure 1

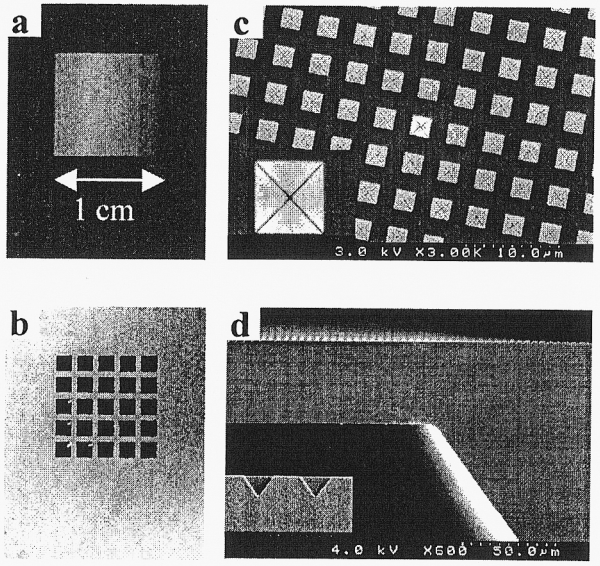

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Figure 2

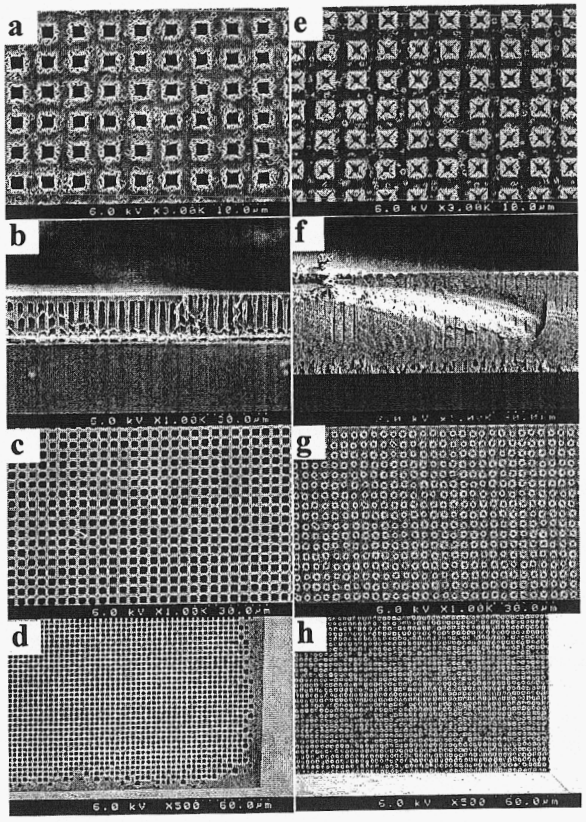
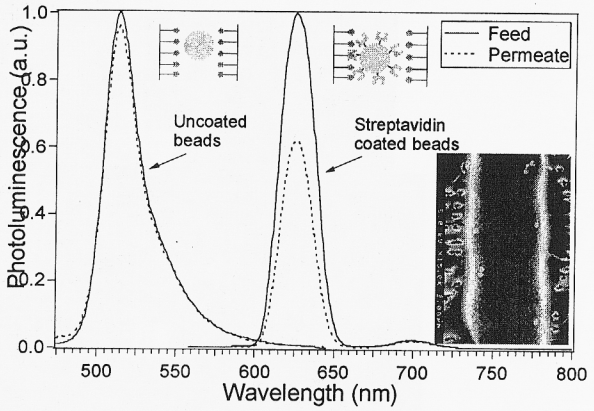


Figure 3


Photoluminescence (a.u.)
1.0
0.8
0.6
0.4
0.2
0.0
500
550
600
650
700
750
800
Wavelength (nm)
Feed
Permeate
Uncoated beads
Streptavidin coated beads